Appendix A

Cell Biology

ATCC® Number:	**HTB-22™** Order this Item	Price:	**£338.00; €498.00**
Designations:	MCF7		
Depositors:	CM McGrath		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial 		
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial		
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Receptors:	estrogen receptor, expressed		
Antigen Expression:	Blood Type O; Rh+		
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15		
Cytogenetic Analysis:	modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.		
Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1		
Age:	69 years adult		
Gender:	female		
Ethnicity:	Caucasian		
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the **WNT7B** oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.		

Related Links ▶

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Material Transfer Agreement

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Related Cell Culture Products

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% .
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
Temperature: 37.0°C

Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

Subculturing:

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time:

29 hrs

Related Products:

purified DNA:ATCC HTB-22D
purified RNA:ATCC HTB-22R
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X
recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

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Appendix B

About the ATCC-LGC Standards Partnership
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ATCC Cultures and Products
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ATCC Product Use Policy

Commercial Use

While ATCC views the MTA as an essential part of distributing its biological materials, the organization readily recognizes the potential utility of ATCC products in commercial applications. To help meet that need, ATCC has set up an out-licensing program that allows companies to use ATCC products for commercial purposes under a non-exclusive licensing agreement. Possible commercial uses of ATCC products that lie outside the scope of the MTA allowed through the ATCC licensing program include:

- Sterility testing
- Vaccine manufacture
- Food & beverage manufacture — yogurt, soda, infant formula
- Product testing
- Clinical diagnostics
- Wastewater treatment and septic additives
- Contract pharmaceutical manufacture
- Drug development
- Contract research — drug discovery, preclinical services, screening
- Medical devices
- Nutraceuticals
- Gene expression databases
- Genome sequencing instrumentation
- Government contracts
- High-throughput technologies
- Proficiency testing
- Research reagents and tools
- Stem cell R&D
- Toxicology testing
- Veterinary pharmaceuticals

Technologies Available for Licensing

Specific ATCC technologies/materials available for commercial licensing agreement are:

- Master cell banks of Vero cells for vaccine manufacture
- Mycoplasma detection kit (version 3)
- Speciation kit
- Materials in ATCC special collections (JHU, National Park Service)
- Pre-1980 cell lines in ATCC collection

The licensing program for commercial use of ATCC products and use of the MTA for ATCC products in research both help protect the ability of ATCC to supply reference standards for the benefit of the scientific community.

For information about commercial use of ATCC materials, contact our Intellectual Property, Licensing and Services Department.

How to Order | Technical Support | ATCC Cultures and Products | ATCC Science
ATCC Standards | ATCC Deposit Services | ATCC Custom Services | ATCC Product Use Policy | Home

Appendix C

Oncogene (2005) 24, 6516–6524

www.nature.com/onc

cFos is critical for MCF-7 breast cancer cell growth

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The activating protein-1 (AP-1) transcription factor is a converging point of multiple signal transduction pathways in many cells. We have previously demonstrated that overexpressing Tam67, a dominant-negative (DN) form of cJun, blocks AP-1 activity and inhibits breast cancer cell growth. We hypothesized that Tam67 forms dimers with other AP-1 proteins to suppress the growth of breast cancer cells. In the present study, we used immunoprecipitation-Western blotting to demonstrate that Tam67 binds all Jun and Fos proteins in breast cancer cells. In addition, we used two variants of the Tam67 mutant to investigate whether Jun or Fos protein was required for breast cancer cell growth. We created a Tam/Fos mutant in which the cJun dimerization domain was replaced by the cFos dimerization domain, and a Tam/Squelcher mutant in which the cJun dimerization domain was deleted. We then isolated MCF-7 cell lines that stably expressed these cJun-DN mutants under the control of an inducible promoter. Using AP-1-dependent reporter assays, we observed that Tam67 and Tam/Fos mutants inhibited AP-1 transcriptional activity, while the Tam/Squelcher mutant did not. We then determined whether Tam/Fos or Tam/Squelcher inhibited breast cell growth as well as Tam67. We found that while Tam67 repressed cell growth, neither Tam/Fos nor Tam/Squelcher mutant affected cell growth. These results indicate that Tam67 likely inactivates Fos family member proteins to suppress breast cancer cell growth. Finally, we performed antisense experiments to knock down the expression of individual family members (cJun or cFos). Our results demonstrated that antisense cFos inhibited breast cancer cell proliferation and colony formation, while antisense cJun did not. These results suggest that Tam67 suppresses breast cancer cell growth by interacting with Fos family members, specifically with cFos, to produce an inactive AP-1 complex.

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Keywords: cFos; cJun; AP-1; breast cancer; cell proliferation

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Introduction

The activating protein-1 (AP-1) transcription factor is a key component of many signal transduction pathways. The AP-1 transcription factor is a dimeric complex consisting of homodimer of Jun proteins (cJun, JunB, or JunD) or heterodimer of Jun and Fos proteins (cFos, FosB, Fra-1, or Fra-2). These heterodimer or homodimer complexes regulate the expression of AP-1 target genes by binding to the AP-1 site (the phorbol 12-*O*-tetradecanoate-13-acetate (TPA)-responsive element) within gene promoters. Other factors such as the Jun dimerization partners (JDP1 and JDP2), the activating transcription factor (ATF)/CRE-binding protein families, Maf proteins, and the neural retina-specific gene product Nrl may also interact with AP-1 proteins and regulate the transcriptional-activating activity of AP-1 complexes (Vogt and Bos, 1990; Angel and Karin, 1991; Aronheim *et al*., 1997; Karin *et al*., 1997; Piu *et al*., 2001). Although the AP-1 factor was identified 15 years ago, the mechanism by which AP-1 and its components control cancer cell proliferation remains unclear. Several mitogenic signaling cascades converge upon the AP-1 factor, making it a potential target for inhibiting breast cell proliferation. Extracellular stimuli such as peptide growth factors and steroid hormones lead to the activation of AP-1 signaling and cell proliferation (Chen *et al*., 1996; Webb *et al*., 1999; Lin *et al*., 2000).

Differential expression and activation of individual Jun and Fos family members allow the AP-1 factor to control a wide variety of cellular functions. AP-1 has been implicated in many different biological processes including cell differentiation, proliferation, and apoptosis (Holt *et al*., 1986; Szabo *et al*., 1991; Brown *et al*., 1993, 1994; Rodgers *et al*., 1994; Ham *et al*., 1995). Extensive analyses of mice and cultured cells have shown that such functional diversity is achieved through the formation of various dimers with different combinations of Jun and Fos proteins.

In breast cancer cells, the AP-1 proteins have been identified as important regulators of growth and invasion. AP-1 proteins are differentially expressed in human breast tumors, and AP-1 activity is modulated by many critical growth factors and hormones such as EGF, IGFs, estrogen, and retinoids (Schule *et al*., 1991; Chen *et al*., 1996; Webb *et al*., 1999; Lin *et al*., 2000). Elevated levels of cJun and phospho-cJun in breast cancer tissue are associated with low estrogen receptor

(ER) expression and tamoxifen resistance (Smith *et al*., 1999; Schiff *et al*., 2000). cJun overexpression in MCF-7 breast cancer cells also produces an invasive and hormone-resistant phenotype (Smith *et al*., 1999). Our previous studies have shown that a specific AP-1 inhibitor, the cJun-dominant negative mutant (cJun-DN), Tam67, blocks AP-1 activity and inhibits breast cancer cell growth *in vitro* and *in vivo* (Brown *et al*., 1994; Ludes-Meyers *et al*., 2001; Liu *et al*., 2002). We also demonstrated that Tam67 causes cell cycle arrest by suppressing G1 cyclin expression and reducing cyclin-dependent kinase activity (Liu *et al*., 2004).

In the present study, we developed three MCF-7 cell lines that express inducible AP-1 mutants (Tam67, Tam/Fos, and Tam/Squelcher) to address the role of Jun and Fos proteins in regulating breast cell growth. Tam67 forms dimer with Jun, Fos, and other cJun-interacting proteins. Tam/Fos is a Tam67 mutant in which the cJun dimerization domain has been replaced by the cFos dimerization domain; thus, it can only heterodimerize with Jun family members to form Tam/Fos : Jun complexes. Tam/Fos lacks the ability to dimerize with cFos or other Fos family member proteins. Tam/Squelcher is a Tam67 mutant that has no dimerization domain, and therefore does not dimerize with any AP-1 family members. We observed that Tam67 inhibited breast cancer cell growth, while Tam/Fos and Tam/Squelcher did not. These results suggest that Fos family members may be inactivated by Tam67. We therefore investigated the role of specific Jun and Fos family members in regulating breast cancer cell growth by performing antisense experiments. Our results demonstrate that cFos is a critical regulator of breast cancer cell growth. These studies will enhance our current understanding of how AP-1 factors function in breast cancer cells and provide a firm rationale for developing selective AP-1 inhibitors for the treatment and prevention of breast cancer.

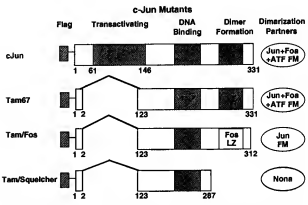


Figure 1 Schematic map of cJun-DN mutants. Tam67 is a cJun-DN mutant in which cJun transactivation domain was deleted. The leucine zipper domain of Tam67 was replaced with that from cFos to produce Tam/Fos. The leucine zipper domain of Tam67 was deleted to produce Tam/Squelcher

Results

Construction of cJun mutants

We developed three cJun mutants by mutating or deleting the functional domains of cJun as shown in Figure 1. These mutants include Tam67 (Jun Δ3–122), Tam/Fos, and Tam/Squelcher. Tam67 is a cJun-DN mutant that can dimerize with Jun, Fos family members, and other interacting proteins (such as ATF proteins). Tam/Fos is a Tam67 mutant in which the cJun dimerization domain has been replaced by the cFos dimerization domain; thus, Tam/Fos is only able to form dimers with Jun family members (no Tam67 : Fos dimers will be formed). Tam/Squelcher is a Tam67 mutant that lacks the cJun dimerization domain and hence lacks the ability to dimerize with any AP-1 family members.

Expression of cJun mutants in MCF-7 cells

Four MCF-7 cell lines (MCF-7 Tet-off Tam67, MCF-7 Tet-off Tam/Fos, MCF-7 Tet-off Tam/Squelcher, and MCF-7 Tet-off vector cells) were generated as described in Materials and methods. Doxycycline (DOX) was removed to induce the expression of cJun mutants, and total protein lysates were prepared 3–7 days after DOX removal. As shown in Figure 2a, each of Tam67 mutants was expressed within 3–5 days after DOX removal. In Figure 2b and c, we show that Tam67 was able to interact with all Jun and Fos proteins using immunoprecipitation (IP)-Western blotting techniques. In Figure 2b, the Tam67 was immunoprecipitated using an anti-Flag antibody, and the resulting precipitated proteins were run on a polyacrylamide gel and screened for specific Jun and Fos proteins. All Jun and Fos proteins were co-immunoprecipitated with Tam67, indicating that Tam67 can bind cJun, JunB, JunD, cFos, FosB, Fra-1, and Fra-2 (Figure 2b). In Figure 2c, the total proteins were immunoprecipitated with individual Jun and Fos antibodies, and the resulting immunoprecipitated proteins were Western blotted using the anti-Flag antibody to determine if Tam67 was present within the protein complexes. Consistent with Figure 2b, Tam67 was shown to be co-immunoprecipitated with each Jun and Fos family members (Figure 2c). Thus, the cJun-DN mutant Tam67 is able to dimerize with all Jun and Fos proteins present in MCF-7 cells.

cJun mutants Tam67 and Tam/Fos suppress basal and heregulin (HRG)-induced AP-1 activity

Tam67 has previously been shown to inhibit AP-1 activity in several different cell lines (Brown *et al*., 1994; Ludes-Meyers *et al*., 2001; Liu *et al*., 2002). In this study, we investigated whether the other cJun mutants, Tam/Fos and TAM/Squelcher, affect AP-1 activity. The MCF-7 Tam/Fos (clones #28 and #73), Tam/Squelcher (clones #25 and #44), and vector-transfected cells (clone #1) were cultured in the presence or absence of DOX to block or induce the expression of Tam/Fos or Tam/Squelcher. As shown in Figure 3, Tam67 and Tam/Fos

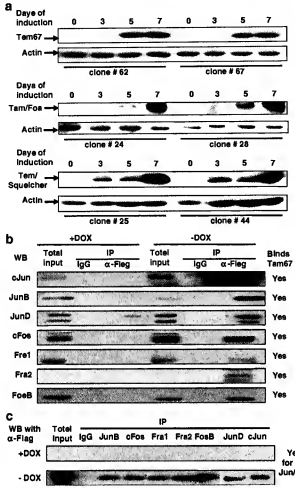


Figure 2 Expression of cJun mutants in MCF-7 Tet-off cells. (**a**) The MCF-7 Tet-off Tam67, MCF-7 Tet-off Tam/Fos, and MCF-7 Tet-off Tam/Squelcher cells were cultured in the medium without DOX for 7 days, and the protein expression was measured by Western blotting. Actin was used as a loading control. (**b**) IP-Western blot to demonstrate that Tam67 was capable of forming dimers with other Jun and Fos proteins. (**c**) IP-Western blot to demonstrate that all Jun and Fos family proteins were able to form dimers with Tam67

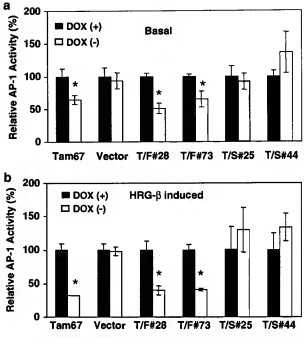


Figure 3 Effect of cJun mutants on AP-1 transcriptional activity. The MCF-7 Tet-off vector, MCF-7 Tet-off Tam67, MCF-7 Tet-off Tam/Fos, and MCF-7 Tet-off Tam/Squelcher cells were cultured in the medium with or without DOX for 7 days to block or induce the expression of cJun mutants. The basal- (**a**) and HRG-β- (**b**) induced AP-1 activities were measured by luciferase assays. *Statistical significance ($P<0.01$)

repressed the basal level of AP-1 activity (Figure 3a) and also inhibited AP-1 activity induced by HRG (Figure 3b) and TPA (data not shown). Tam/Squelcher did not affect basal or induced AP-1 activity (Figure 3a and b).

Effect of cJun mutants on breast cancer cell growth

In previous studies, we demonstrated that Tam67 inhibits breast cancer cell growth (Ludes-Meyers *et al*., 2001; Liu *et al*., 2002). For the present study, we wanted to identify the dimer partners of Tam67 that when inactivated by Tam67 lead to growth suppression. The MCF-7 Tet-off Tam/Fos (clones #28 and #24), Tam/Squelcher (clones #25 and #44), and vector cells (clone #1) were cultured in the presence or absence of DOX for 7 days to suppress or induce the expression of Tam/Fos or Tam/Squelcher. Cell proliferation was then measured. As shown in Figure 4, Tam67 suppressed breast cancer cell growth in the absence of DOX. However, while Tam/Fos inhibited AP-1 activity (as defined by activity of collagenase reporter construct and shown in Figure 3), it did not suppress cell growth. This result suggests that suppression of AP-1 transcriptional-activating activity alone is not sufficient to suppress breast cancer growth. Tam/Squelcher (which failed to suppress AP-1 transcriptional-activating activity) also did not inhibit breast cancer cell growth. These results suggest that Tam67 must dimerize with factors other than Jun family members to inhibit breast cancer cell growth. Thus, we hypothesized that Tam67 inactivates Fos to suppress cell growth.

Antisense cJun and cFos inhibit AP-1 activity

To determine whether cFos is required for breast cancer growth, we used antisense cDNA to inhibit cJun and cFos expression in the breast cancer cells. MCF-7 cells were transfected with antisense cJun, antisense cFos, or pcDNA3.1 vector. We first verified that cJun and cFos protein expression was suppressed by antisense cJun or cFos cDNA. As shown in Figure 5, antisense constructs effectively reduced cJun or cFos protein expression. We then performed luciferase reporter assays using an

AP-1-dependent reporter construct to determine the effect of antisense cJun and antisense cFos on AP-1 activity. As shown in Figure 6, we observed that both antisense cJun and antisense cFos reduced AP-1 transcriptional-activating activity.

Antisense cFos suppresses breast cancer cell growth

We next used a single-cell proliferation assay previously described by us (Ludes-Meyers *et al.*, 2001) to investigate the importance of cJun and cFos on the growth of breast cancer cells. In this assay, shown schematically in Figure 7a, cells were transfected with a β-galactosidase plasmid along with Tam67, antisense cJun, antisense cFos constructs, or vector. The transfected cells were then split and plated as single cells that were then allowed to grow for three to five doublings to form colonies. The cells were then stained with X-gal to identify transfected cells (cells positive for β-galactosidase, stained blue). Cells stained blue are progeny from one transfected cell. The number of β-galactosidase-positive progeny cells in each stained colony was then counted to assay the growth of the single transfected cells. The numbers of progeny cells (number of blue cells in a colony) were then plotted as a histogram. This assay allowed us to determine the effect of knocking down cJun or cFos expression on the growth of individual transfected cells.

Our results showed that after 3 days, Tam67 dramatically reduced the number of cells that grew from a single transfected cell as compared to vector-transfected cells. This degree of growth suppression was similar to that seen when antisense cFos was transfected. Alternatively, the colony distribution of vector control and antisense cJun showed similar patterns (Figure 7b). Thus, cFos knockdown with antisense cDNA significantly suppressed the growth of breast cancer cells ($P<0.0001$).

We next confirmed the results of our single-cell growth assay by performing a colony formation assay in cells stably transfected with antisense cJun and antisense cFos cDNA. In these experiments, we stably transfected cJun, and cFos antisense cDNAs into MCF-7 cells, and measured the colony formation of the resulting clones. As shown in Figure 7c, the vector-transfected cells produced 476 ± 65 colonies, cells transfected with cJun antisense cDNA formed 386 ± 75 colonies, and cFos antisense-transfected cells formed 304 ± 14 colonies. Thus, antisense cFos significantly reduced the ability of MCF-7 cells to form colonies, compared to the vector control ($P<0.01$). These results are consistent with our results from the single-cell proliferation assay (shown in Figure 7b), and demonstrate that cFos is critical for breast cancer cell growth. From these results, we conclude that cFos is an important regulator of MCF-7 breast cancer cell proliferation that may play a critical role in the regulation of human breast cancers.

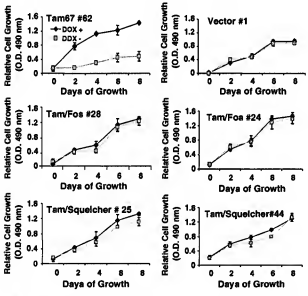


Figure 4 Effect of cJun mutants on MCF-7 cell proliferation. The MCF-7 Tet-off vector, MCF-7 Tet-off Tam67, MCF-7 Tet-off Tam/Fos, and MCF-7 Tet-off Tam/Squelcher cells were cultured in the medium with or without DOX for 7 days to block or induce the expression of cJun mutants. Cell proliferation was measured over the next 8 days using MTS assay

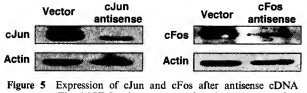


Figure 5 Expression of cJun and cFos after antisense cDNA treatment. The MCF-7 cells were transfected with pcDNA3.1, pcNDA3.1 antisense cJun, or antisense cFos. After 2 weeks of selection, the whole-cell lysates from transfected cells were used to measure cJun and cFos protein expression by Western blotting

Discussion

We have previously shown that the cJun-DN mutant Tam67 blocks AP-1 transcriptional activity and inhibits

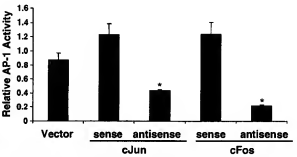
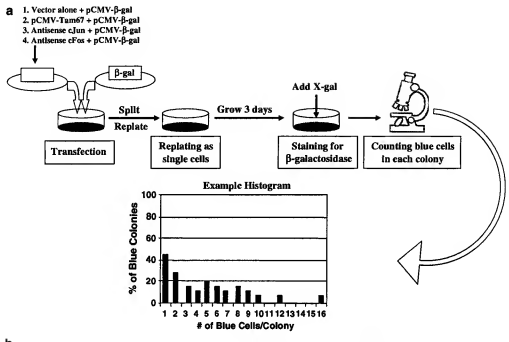
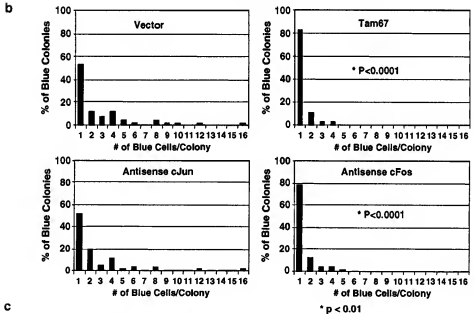
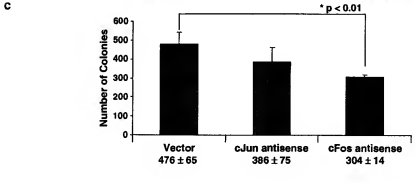


Figure 6 Effect of antisense cJun and cFos cDNA on AP-1 activity. The MCF-7 cells were transfected with pcDNA3.1 alone or pcDNA3.1 antisense cJun or antisense cFos. After 48 h, the cells were lysed and AP-1 activity was measured by luciferase assays. *Statistical significance ($P<0.005$) between the effect of vector and antisense cJun or cFos


a
1. Vector alone + pCMV-β-gal
2. pCMV-Tam67 + pCMV-β-gal
3. Antisense cJun + pCMV-β-gal
4. Antisense cFos + pCMV-β-gal
β-gal
Transfection
Split
Replate
Replating as single cells
Grow 3 days
Add X-gal
Staining for β-galactosidase
Counting blue cells in each colony
Example Histogram
% of Blue Colonies
# of Blue Cells/Colony


b
Vector
Tam67
* P<0.0001
Antisense cJun
Antisense cFos
* P<0.0001
% of Blue Colonies
# of Blue Cells/Colony


c
* p < 0.01
Number of Colonies
Vector
476 ± 65
cJun antisense
386 ± 75
cFos antisense
304 ± 14

breast cancer cell growth *in vitro* and *in vivo* (Brown *et al*., 1994, 1996; Ludes-Meyers *et al*., 2001; Liu *et al*., 2002). This cJun mutant dimerizes with Jun or Fos proteins to inhibit AP-1 activity and suppress the expression of AP-1-regulated genes involved in cell growth (Brown *et al*., 1994, 1996; Hennigan and Stambrook, 2001). The mechanism by which AP-1 blockade suppresses breast cell growth is not entirely clear, and is the focus of this study. We used three cJun mutants, and antisense cDNA for cJun and cFos to elucidate the underlying mechanism by which Tam67 suppresses cell growth. Since Tam67 is able to interact with Jun, Fos, and other factors, we diminished the binding capacity of Tam67 by replacing the dimerization domain with that from cFos. This produces a cJun mutant that only forms Tam:Jun family dimers. We also created the Tam/Squelcher mutant that is unable to bind any AP-1 proteins. We then tested whether these mutants could suppress the growth of MCF-7 breast cancer cells as effectively as Tam67 does. We found that the Tam/Fos mutant, which only forms complexes with Jun proteins, did not suppress MCF-7 cell growth, despite being able to decrease basal and induced AP-1 transcriptional activity. These results suggest that cFos or other Fos family member proteins must be inactivated by Tam67 to suppress cell proliferation. Our results also show that molecules that suppress AP-1 activity as measured by a collagenase reporter assay do not necessarily suppress breast cancer cell growth. This result suggests that it will be more relevant to screen growth-suppressive anti-AP-1 agents using AP-1-dependent promoters that are more directly related to growth. Such AP-1-dependent, growth-regulatory genes include cyclin D1 and c-Myc. We have recently demonstrated that these important growth-regulatory genes are critically regulated by AP-1 in breast cancer cells (DeNardo *et al*., 2005).

To further investigate the role of Fos family members in regulating growth, we used antisense strategies to knockdown cJun or cFos mRNA and protein. We observed that cFos antisense suppresses breast cancer cell growth, while antisense cJun does not. From these studies, we conclude that cFos is a critical mediator of AP-1-regulated breast cancer cell growth.

AP-1 transcription factors are involved in cellular proliferation (Holt *et al*., 1986), differentiation (Szabo *et al*., 1991), apoptosis (Ham *et al*., 1995), oncogene-induced transformation (Brown *et al*., 1993, 1994), invasion (Angel *et al*., 1987; McDonnell *et al*., 1990; Mackay *et al*., 1992; Matrisian, 1994), and several other cellular functions (Chiu *et al*., 1989; Schutte *et al*., 1989; Yoshioka *et al*., 1995). Jun and Fos members are differentially expressed in human breast cancers (Bamberger *et al*., 1999), and AP-1 is activated by important growth factors including EGF, IGFs, and estrogen (Chen *et al*., 1996; Ludes-Meyers *et al*., 2001; Liu *et al*., 2002). Elevated levels of cJun and phospho-cJun in human breast cancers are associated with low ER expression (Tang *et al*., 1997; Bamberger *et al*., 1999; Gee *et al*., 2000) and with tamoxifen resistance (Daschner *et al*., 1999; Johnston *et al*., 1999; Schiff *et al*., 2000; Liu *et al*., 2004). In addition, cJun overexpression in MCF-7 cells produces a tumorigenic-, invasive-, and hormone-resistant phenotype (Smith *et al*., 1999; Gee *et al*., 2000; Schiff *et al*., 2000). All of these previous studies suggest that AP-1 plays a critical role in the breast cell growth and transformation.

As a member of the AP-1 transcription factor complex, cFos is an essential modulator of cell proliferation, differentiation, and transformation (Preston *et al*., 1996). Depending on the cell type and environment, cFos can function either as a transcriptional activator or as a transcriptional repressor. Transition between these functions is regulated through post-translational modifications of the C-terminal region of the protein, possibly by phosphorylation of the serine residues (Barber and Verma, 1987). Earlier studies demonstrated that antisense RNA or Fos-specific antibodies that block cFos expression inhibited fibroblasts proliferation (Holt *et al*., 1986; Nishikura and Murray, 1987; Riabowol *et al*., 1988). Our current results in breast cells are consistent with these previous studies. The specific role of different Fos family proteins is still undefined, although each of the four Fos proteins may be required for cellular proliferation (Piechaczyk and Blanchard, 1994). In breast cancer, increased cFos protein expression was associated with poor prognosis (Bland *et al*., 1995). Knockdown of cFos expression was able to prolong survival and inhibit the proliferation and invasiveness of breast cancer xenografts (Robinson-Benion *et al*., 1994; Arteaga and Holt, 1996). Furthermore, Gee *et al*. (1995) have observed a significant association between elevated Fos protein expression and increased proliferation, *de novo* endocrine insensitivity, and a poor prognosis in clinical breast cancers. In addition to its role in normal development and cellular growth, the cFos protein is associated with apoptotic

Figure 7 Cell proliferation assays of MCF-7 breast cancer cells. **(a)** Schematic diagram of the single-cell proliferation assay. MCF-7 cells were cotransfected with pCMV-β-Gal (0.2 μg) and either pcDNA3.1 vector (2 μg), pcDNA3.1 Tam67 (2 μg), pcDNA3.1 antisense cJun cDNA (2 μg), or pcDNA3.1 antisense cFos cDNA (2 μg). After 3 days of growth (approximately three doublings), the transfected cells were identified by staining *in situ* with X-gal for β-galactosidase activity, and the number of transfected cells per colony was counted. The results are shown as histograms of one to 16 stained cells per colony. Colonies with same numbers of blue cells are shown as a percentage of the total stained colonies. **(b)** Antisense cFos inhibits single-cell proliferation in MCF-7 cells. MCF-7 cells were treated as described above. The statistical significance of these results was analysed using Wilcoxon's rank-sum test. The *P*-values from Wilcoxon's rank-sum test are shown. **(c)** Antisense cFos cDNA reduces colony formation in MCF-7 cells. MCF-7 cells were transfected with pcDNA3.1 alone or pcNDA3.1 antisense cJun or antisense cFos. After 2 weeks of selection with G418, survived colonies were stained with crystal violet and counted. The statistical significance of these results was analysed using two-sample *t*-tests

cell death in antiproliferative conditions, and in response to cellular injury (Preston *et al.*, 1996). However, the proapoptotic role of cFos is not evident in *c-fos* knockout mice (Gajate *et al.*, 1996; Roffler-Tarlov *et al.*, 1996). Our studies support these data by indicating that cFos is involved in mammary cell proliferation and transformation. It appears that cFos is an important activator of the AP-1-regulated genes involved in growth.

While our results show that cFos is a critical AP-1 protein involved in regulating breast cell proliferation, other AP-1 family members within the mammary glands may regulate breast cell proliferation as well. Other factors such as FosB, Fra-1, Fra-2, or ATF proteins (all of which can dimerize with cJun) may also be important for breast cell growth. We are currently investigating the role of those AP-1-related proteins on breast cell growth.

In our previous studies, we have seen that Tam67 effectively inhibited the growth of normal, immortal breast cells, and ER-positive breast cancer cells, but not of ER-negative breast cancer cells (Ludes-Meyers *et al.*, 2001). These previous results suggest that some breast cancer cells do not require AP-1 for their growth. It will be interesting to explore whether cFos has a similar role in these breast cancer cells. Such differential sensitivity of these breast cells to AP-1 blockade indicates that cFos is a critical factor in only a subset of breast cancers.

Based on our results, we propose that suppression of AP-1 activity by Tam67 leads to cFos inactivation, which in turn causes suppression of breast cancer growth by modulating AP-1-dependent genes. We are currently attempting to identify the critical growth-regulatory genes that are affected by cFos inactivation. Such studies will lead to a better understanding of how AP-1 controls proliferation, and may ultimately uncover new targets for the treatment and prevention of breast cancer.

Materials and methods

Plasmids

The Tam67, Tam/Fos, and Tam/Squelcher genes were constructed using the polymerase chain reaction(PCR) as described previously (Alani *et al.*, 1991; Brown *et al.*, 1994, 1996). The correct sequence of these fusion genes was confirmed by sequencing using an automated DNA sequencer. All cJun mutants were cloned into pUHD 10-3 5′ Flag vector for further use.

Cell culture, transfection, and cell lines established

The generation of MCF-7 Tet-off Tam67 clones has been described previously (Ludes-Meyers *et al.*, 2001). The cells were maintained in Improved MEM (high zinc option, Life Technologies, Grand Island, NY, USA), with 100 μg/ml of geneticin and 100 μg/ml hygromycin in the presence of DOX. The MCF-7 Tet-off parental cells were maintained in Improved MEM (high zinc option, Life Technologies, Grand Island, NY, USA) with 100 μg/ml of geneticin. The cells were transfected with Tam67, Tam/Fos, Tam/Squelcher, or vector alone using Fugene 6 reagent (Roche, Indianapolis, ID, USA) according to the manufacturer's recommendations. Stable clones were isolated after selection in hygromycin.

Luciferase assay to measure AP-1 activity

AP-1 transcriptional activity in cells was measured using the Dual-Luciferase™ Reporter Assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. The cells were cotransfected with the Col-Z-Luc reporter gene containing the luciferase gene linked to 1100 bp of the human collagenase gene promoter that contains a single AP-1 binding site (TGAG/CTCA) and pRL-TK, a *Renilla* construct for normalizing transfection efficiency. To determine the AP-1 activity stimulated by heregulin-beta (HRG-β), the cells were treated with HRG-β (10 ng/ml, R&D System, Minneapolis, MO, USA) or DMSO, respectively, for 6 h before harvest. Transfected cells were lysed 36 h after transfection and luciferase activity was measured with equal amounts of cell extract using a microplate luminometer (Labsystems, Helsinki, Finland) and normalized to *Renilla* activity.

Single-cell proliferation assay

This assay was performed as described previously (Ludes-Meyers *et al.*, 2001). Briefly, MCF-7 cells were cotransfected with 0.2 μg of pCMV-β-Gal and 2 μg of either pcDNA3.1 vector, pcDNA3.1 TAM67, pcDNA3.1 antisense cJun cDNA, or pcDNA3.1 antisense cFos cDNA. At 12 h after transfection, the cells were trypsinized to make single-cell suspensions, and split into 100 mm dishes. After 3 days, colonies of cells were fixed and stained with X-gal to detect cells expressing β-galactosidase *in situ*. Colonies containing stained, blue cells were visualized under a light microscope and scored for the total number of stained colonies and the number of blue cells per colony. The cells in these blue colonies received DNA and arose from a single transfected cell. Therefore, transfection efficiency in these counted blue colonies is 100%. The number of blue cells per colony is a function of the growth rate of a single transfected cell. The results were then plotted as the % colonies that showed one to 16 stained cells per colony. Transfected genes that cause decreased growth reduce the number of blue cells within individual colonies.

Colony formation assay

This assay was performed as described previously (Ludes-Meyers *et al.*, 2001). Briefly, 2×10^5 cells were cotransfected in a six-well plate with 2 μg of either pcDNA3.1 empty vector, pcDNA3.1 antisense cJun, or pcDNA3.1 antisense cFos. At 12 h after transfection, the cells were split and maintained in 100 mm dishes. At 24 h after transfection, G418 (Invitrogen) was added to make a final concentration of 800 μg/ml. After 2 weeks of selection in G418, resistant colonies were stained with crystal violet and counted under a light microscope. All experiments were performed in triplicate and the mean numbers of G418-resistant colonies were calculated.

Cell proliferation assay of stably transfected Tet-off cell lines

The CellTiter 96™ AQueous Non-Radioactive Cell Proliferation Assay ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazo-lium) (MTS) assay; Promega, Madison, WI, USA) was used to measure breast cancer cell growth according to the protocol provided by the manufacturer. Approximately 1000 cells were seeded in a 96-well plate. A solution containing a 20:1 ratio of MTS and PMS (phenazine methosulfate) was added to the cells for 2 h at

37°C and absorption at 550 nm was determined. Each data point was performed in quadruplet, and the results were reported as mean absorption ± s.e.

Western blot assay

Cells were transfected with antisense cDNA for cJun, cFos, or vector alone. After 36 h, whole-cell lysates were extracted. Then, equal amounts of total protein were electrophoresed on a 10% acrylamide denaturing gel and transferred onto a nitrocellulose membrane. The cJun or cFos expression was detected by using the following antibodies: rabbit polyclonal antibody specific for cJun (cat.#: PC06, Oncogene Science, Cambridge, MA, USA; 1 : 200); mouse monoclonal antibody specific for cFos (cat.#: sc-7202, Santa Cruz, CA, USA; 1 : 200).

IP-Western blot

MCF-7 Tet-off TAM67 cells were maintained as described above in the Cell Culture section. Induction of TAM67 was obtained by withdrawal of DOX for 3–5 days. Then, total cell lysates were collected. Lysates with equal amounts of total protein were precleared with 50 μl of protein G-agarose for 30 min at 4°C and then incubated overnight with 1 μg of antibodies against individual Jun and Fos proteins. All antibodies were procured from Santa Cruz Biotech Inc. (Santa Cruz, CA, USA). Protein G-agarose was added for another 4 h and the beads were pelleted and washed three times with PBS. Bound proteins were eluted in SDS sample buffer, subjected to SDS–PAGE, and analysed by regular Western blot as described above.

Statistical analysis

The results of colony formation assays were expressed as mean number of colonies ± s.e. Statistical significance was determined using the Student's *t*-test. Single-cell proliferation assays produced results that showed a distribution of cells per colony. For this assay, Wilcoxon's rank-sum tests were used to compare distributions between antisense cJun- and vector-transfected cells, or antisense cFos- and vector-transfected cells.

Abbreviations

ATF, activating transcription factor; AP-1, activating protein-1; Tam67, cJun dominant-negative mutant; DOX, doxycycline; HRG-β, heregulin-beta; TPA, phorbol 12-*O*-tetradecanoate-13-acetate.

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Appendix D

Cell culture

From Wikipedia, the free encyclopedia

Cell culture is the process by which cells are grown under controlled conditions. In practice the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture.

Animal cell culture became a common laboratory technique in the mid-1900s,[1] but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century.[2]

Epithelial cells in culture, stained for keratin (red) and DNA (green)

Contents

History

The 19th-century English physiologist Sydney Ringer developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside of the body.[1] (http://www.whonamedit.com/synd.cfm/2119.html) In 1885 Wilhelm Roux removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture.[3] Ross Granville Harrison, working at Johns Hopkins Medical School and then at Yale University, published results of his experiments from 1907-1910, establishing the methodology of tissue culture.[4]

Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The Salk polio vaccine was one of the first products mass-produced using cell culture techniques. This vaccine was made possible by the cell culture research of John Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins, who were awarded a Nobel Prize for their discovery of a method of growing the virus in monkey kidney cell cultures.

Concepts in mammalian cell culture

Isolation of cells

Cells can be isolated from tissues for *ex vivo* culture in several ways. Cells can be easily purified from blood, however only the white cells are capable of growth in culture. Mononuclear cells can be released from soft tissues by *enzymatic digestion* with enzymes such as collagenase, trypsin, or pronase, which break down the extracellular matrix. Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture. This method is known as *explant culture*.

Cells that are cultured directly from a subject are known as ***primary cells***. With the exception of some derived from tumors, most primary cell cultures have limited lifespan. After a certain number of population doublings cells undergo the process of senescence and stop dividing, while generally retaining viability.

An established or **immortalised** *cell line* has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification, such as artificial expression of the telomerase gene. There are numerous well established cell lines representative of particular cell types.

Maintaining cells in culture

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO_2 for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. The growth factors used to supplement media are often derived from animal blood, such as calf serum. One complication of these blood-derived ingredients is the potential for contamination of the culture with viruses or prions, particularly in biotechnology medical applications. Current practice is to minimize or eliminate the use of these ingredients wherever possible, but this cannot always be accomplished.

Cells can be grown in *suspension* or *adherent* cultures. Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the bloodstream. There are also cell lines that have been modified to be able to survive in suspension cultures so that they can be grown to a higher density than adherent conditions would allow. Adherent cells require a surface, such as tissue culture plastic, which may be coated with extracellular matrix components to increase adhesion properties and provide other signals needed for growth and differentiation. Most cells derived from solid tissues are adherent. Another type of adherent culture is *organotypic culture* which involves growing cells in a three-dimensional environment as opposed to two-dimensional culture dishes. This 3D culture system is biochemically and physiologically more similar to *in vivo* tissue, but is technically challenging to maintain because of many factors (e.g. diffusion).

Cell line cross-contamination

Cell line cross-contamination can be a problem for scientists working with cultured cells. Studies suggest that anywhere from 15–20% of the time, cells used in experiments have been misidentified or contaminated with another cell line.[5][6][7] Problems with cell line cross contamination have even been detected in lines from the NCI-60 panel, which are used routinely for drug-screening studies. [8][9] Major cell line repositories including the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ) have received cell line submissions from researchers that were misidentified by the researcher.[8][10] Such contamination poses a problem for the quality of research produced using cell culture lines, and the major repositories are now authenticating all cell line submissions.[11] ATCC uses short tandem repeat (STR) DNA fingerprinting to authenticate its cell lines.[12]

To address this problem of cell line cross-contamination, researchers are encouraged to authenticate their cell lines at an early passage to establish the identity of the cell line. Authentication should be repeated before freezing cell line stocks, every two months during active culturing and before any publication of research data generated using the cell lines. There are many methods for identifying cell lines including isoenzyme analysis, human lymphocyte antigen (HLA) typing and STR analysis.[12]

Manipulation of cultured cells

As cells generally continue to divide in culture, they generally grow to fill the available area or volume. This can generate several issues:

- Nutrient depletion in the growth media
- Accumulation of apoptotic/necrotic (dead) cells.
- Cell-to-cell contact can stimulate cell cycle arrest, causing cells to stop dividing known as contact inhibition or senescence.
- Cell-to-cell contact can stimulate cellular differentiation.

Among the common manipulations carried out on culture cells are media changes, passaging cells, and transfecting cells. These are generally performed using tissue culture methods that rely on sterile technique. Sterile technique aims to avoid contamination with bacteria, yeast, or other cell lines. Manipulations are typically carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms. Antibiotics (e.g. penicillin and streptomycin) and antifungals (e.g. Amphotericin B) can also be added to the growth media.

As cells undergo metabolic processes, acid is produced and the pH decreases. Often, a pH indicator is added to the medium in order to measure nutrient depletion.

Media changes

In the case of adherent cultures, the media can be removed directly by aspiration and replaced.

Passaging cells

Passaging (also known as subculture or splitting cells) involves transferring a small number of cells into a new vessel. Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density. Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media. For adherent cultures, cells first need to be detached; this is commonly done with a

mixture of trypsin-EDTA, however other enzyme mixes are now available for this purpose. A small number of detached cells can then be used to seed a new culture.

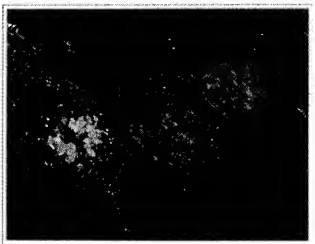
Transfection and transduction

Another common method for manipulating cells involves the introduction of foreign DNA by transfection. This is often performed to cause cells to express a protein of interest. More recently, the transfection of RNAi constructs have been realized as a convenient mechanism for suppressing the expression of a particular gene/protein.

DNA can also be inserted into cells using viruses, in methods referred to as transduction, infection or transformation. Viruses, as parasitic agents, are well suited to introducing DNA into cells, as this is a part of their normal course of reproduction.

Established human cell lines

Cell lines that originate with humans have been somewhat controversial in bioethics, as they may outlive their parent organism and later be used in the discovery of lucrative medical treatments. In the pioneering decision in this area, the Supreme Court of California held in Moore v. Regents of the University of California that human patients have no property rights in cell lines derived from organs removed with their consent. [13]



One of the earliest human cell lines, descended from Henrietta Lacks, who died of the cancer that those cells originated from, the cultured HeLa cells shown here have been stained with Hoechst turning their nuclei blue.

Generation of hybridomas

For more details on this topic, see Hybridoma.

It is possible to fuse normal cells with an immortalised cell line. This method is used to produce monoclonal antibodies. In brief, lymphocytes isolated from the spleen (or possibly blood) of an immunised animal are combined with an immortal myeloma cell line (B cell lineage) to produce a hybridoma which has the antibody specifity of the primary lymphoctye and the immortality of the myeloma. Selective growth medium (HA or HAT) is used to select against unfused myeloma cells; primary lymphoctyes die quickly in culture and only the fused cells survive. These are screened for production of the required antibody, generally in pools to start with and then after single cloning.

Applications of cell culture

Mass culture of animal cell lines is fundamental to the manufacture of viral vaccines and many products of biotechnology. Biological products produced by recombinant DNA (rDNA) technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals (monoclonal antibodies, interleukins, lymphokines), and anticancer agents. Although many simpler proteins can be produced using rDNA in bacterial cultures, more complex proteins that are glycosylated (carbohydrate-modified) currently must be made in animal cells. An important example of such a complex protein is the hormone erythropoietin. The cost of growing mammalian cell cultures is high, so research is underway to produce such complex proteins in insect cells or in higher plants.

Tissue culture and engineering

Cell culture is a fundamental component of tissue culture and tissue engineering, as it establishes the basics of growing and maintaining cells *ex vivo*.

Vaccines

Vaccines for polio, measles, mumps, rubella, and chickenpox are currently made in cell cultures. Due to the H5N1 pandemic threat, research into using cell culture for influenza vaccines is being funded by the United States government. Novel ideas in the field include recombinant DNA-based vaccines, such as one made using human adenovirus (a common cold virus) as a vector,[14][15] or the use of adjuvants. [16]

Culture of non-mammalian cells

Plant cell culture methods

See also: Tobacco BY-2 cells

Plant cell cultures are typically grown as cell suspension cultures in liquid medium or as callus cultures on solid medium. The culturing of undifferentiated plant cells and calli requires the proper balance of the plant growth hormones auxin and cytokinin.

Bacterial/Yeast culture methods

For bacteria and yeast, small quantities of cells are usually grown on a solid support that contains nutrients embedded in it, usually a gel such as agar, while large-scale cultures are grown with the cells suspended in a nutrient broth.

Viral culture methods

The culture of viruses requires the culture of cells of mammalian, plant, fungal or bacterial origin as hosts for the growth and replication of the virus. Whole wild type viruses, recombinant viruses or viral products may be generated in cell types other than their natural hosts under the right conditions. Depending on the species of the virus, infection and viral replication may result in host cell lysis and formation of a viral plaque.

Common cell lines

Human cell lines

- National Cancer Institute's 60 cancer cell lines
- ESTDAB database http://www.ebi.ac.uk/ipd/estdab/directory.html

- DU145 (Prostate cancer)
- Lncap (Prostate cancer)
- MCF-7 (breast cancer)
- MDA-MB-438 (breast cancer)
- PC3 (Prostate cancer)
- T47D (breast cancer)
- THP-1 (acute myeloid leukemia)
- U87 (glioblastoma)
- SHSY5Y Human neuroblastoma cells, cloned from a myeloma
- Saos-2 cells (bone cancer)

Primate cell lines

- Vero (African green monkey *Chlorocebus* kidney epithelial cell line initiated 1962)

Rat tumor cell lines

- GH3 (pituitary tumor)
- PC12 (pheochromocytoma)

Mouse cell lines

- MC3T3 (embryonic calvarial)

Plant cell lines

- Tobacco BY-2 cells (kept as cell suspension culture, they are model system of plant cell)

Other species cell lines

- zebrafish ZF4 and AB9 cells.
- *Madin-Darby Canine Kidney (MDCK)* epithelial cell line
- Xenopus A6 kidney epithelial cells.

List of cell lines

Cell line	Meaning	Organism	Origin tissue	Morphology	
293-T		Human	kidney		Derivative of HEK 293ECACC (http://www.hpacultures.org

			(embryonic)		
3T3 cells	"3-day transfer, inoculum 3 x 105 cells"	Mouse	embryonic fibroblast		Also known as NIH 3T3 ECACC (http://www.hpacultures.o
721		Human	melanoma		
9L		Rat	glioblastoma		
A2780		Human	Ovary	Ovarian Cancer	ECACC (http://www.hpacultures.org.uk/products/celllines/g
A2780ADR		Human	Ovary	Adriamycin-resistant derivative	ECACC (http://www.hpacultures.org.uk/products/celllines/g
A2780cis		Human	Ovary	Cisplatin-resistant derivative	ECACC (http://www.hpacultures.org.uk/products/celllines/g
A172		human	glioblastoma	malignant glioma	ECACC (http://www.hpacultures.org.uk/products/celllines/g
A20		murine	B lymphoma	B lymphocyte	
A253		human	head and neck carcinoma	submandibular duct	
A431		human	skin epithelium	squamous carcinoma	ECACC (http://www.hpacultures.org.uk/products/celllines/g
A-549		human	lungcarcinoma	epithelium	DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/dsmz_nr=107&from=cell_line_index&select=A&term=&pr (http://www.hpacultures.org.uk/products/celllines/generalcel
ALC		murine	bone marrow	stroma	NCBI (http://www.ncbi.nlm.nih.gov/pubmed/2435412)
B16		murine	Melanoma		ECCAC (http://www.hpacultures.org.uk/products/celllines/g
B35		rat	Neuroblastoma		ATCC (http://www.lgcpromochem-atcc.com/common/catalo
BCP-1 cells		Human	PBMC	HIV+ lymphoma	ATCC (http://www.atcc.org/common/catalog/numSearch/nu
bEnd.3	*brain endothelial*	mouse	brain / cerebral Cortex	endothelium	ATCC (http://www.lgcpromochem-atcc.com/common/catalo
BHK-21	"Baby Hamster Kidney Fibroblast cells"	Hamster	kidney	fibroblast	ECACC (http://www.hpacultures.org.uk/products/celllines/g (http://www.olympusmicro.com/primer/techniques/fluoresce
BR 293		human	breast	breast cancer	
BxPC3	Biopsy xenograph of pancreatic carcinoma line 3	human	pancreatic adenocarcinoma	epithelial	ATCC (http://www.lgcpromochem-atcc.com/common/catalo
C3H-10T1/2		Mouse	Embryonic mesenchymal cell line		ECACC (http://www.hpacultures.org.uk/products/celllines/g
C6/36		Asian tiger mosquito	larval tissue		ECACC (http://www.hpacultures.org.uk/products/celllines/g
Cal-27		human	tongue	squamous cell carcinoma	
CHO	*Chinese hamster ovary*	hamster	Ovary	epithelium	ECACC (http://www.hpacultures.org.uk/products/celllines/g line_id=724&x=23&y=17)
COR-L23		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
COR-L23/CPR		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
COR-L23/5010		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
COR-L23/R23		Human	Lung	Epithelial	ECACC (http://www.hpacultures.org.uk/products/celllines/g
COS-7	*Cercopithecus aethiops, origin-defective SV-40*	ape - *Cercopithecus aethiops* (Chlorocebus)	kidney	fibroblast	ECACC (http://www.hpacultures.org.uk/products/celllines/g atcc.com/common/catalog/numSearch/numResults.cfm?atcc
CML T1	*Chronic Myelod Leukaemia T-lymphocyte 1*	human	CML acute phase	T cell leukaemia	Blood (http://bloodjournal.hematologylibrary.org/cgi/reprint/
CMT	*canine mammary tumor*	dog	mammary gland	epithelium	
CT26		murine	Colorectal Carcinoma	Colon	

D17		canine	osteosarcoma		ECACC (http://www.hpacultures.org.uk/products/celllines/g
DH82		canine	histiocytosis	monocyte/macrophage	ECACC (http://www.hpacultures.org.uk/products/celllines/g J Vir Meth (http://www.ingentaconnect.com/content/els/016(
DU145		human	Androgen insensitive carcinoma	Prostate	
DuCaP	Dura mater Cancer of the Prostate	human	Metastatic Prostate Cancer	epithelial	PubMed (http://www.ncbi.nlm.nih.gov/pubmed/11317521?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubm
EL4		mouse		T cell leukaemia	ECACC (http://www.hpacultures.org.uk/products/celllines/g
EM2		human	CML blast crisis	Ph+ CML line	Cell Line Data Base (http://bioinformatics.istge.it/hypercldb/
EM3		human	CML blast crisis	Ph+ CML line	Cell Line Data Base (http://bioinformatics.istge.it/hypercldb/
EMT6/AR1		mouse	Breast	Epithelial-like	ECACC (http://www.hpacultures.org.uk/products/celllines/g
EMT6/AR10.0		Mouse	Breast	Epithelial-like	ECACC (http://www.hpacultures.org.uk/products/celllines/g
FM3		human	Metastatic lymph node	melanoma	
H1299		human	lung	lung cancer	
H69		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
HB54		hybridoma	hybridoma	secretes L243 mAb (against HLA-DR)	Human Immunology (http://www.sciencedirect.com/science?D&_user=2471587&_rdoc=1&_fmt=&_orig=search&_sort=
HB55		hybridoma	hybridoma	secretes MA2.1 mAb (against HLA-A2 and HLA-B17)	Journal of Immunology (http://www.jimmunol.org/cgi/reprir
HCA2		human	fibroblast		Journal of General Virology (http://vir.sgmjournals.org/cgi/c
HEK-293	*human embryonic kidney*	human	kidney (embryonic)	epithelium	ATCC (http://www.lgcpromochem-atcc.com/common/catalo
HeLa	*Henrietta Lacks*	human	Cervical cancer	epithelium	DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/dsmz_nr=57&from=cell_line_index&select=H&term=&pres (http://www.hpacultures.org.uk/products/celllines/generalcel
Hepa1c1c7	clone 7 of clone 1 hepatoma line 1	mouse	Hepatoma	epithelial	ECACC (http://www.hpacultures.org.uk/products/celllines/g ATCC (http://www.lgcpromochem-atcc.com/common/catalo
HL-60	*human leukemia*	human	Myeloblast	bloodcells	ECACC (http://www.hpacultures.org.uk/products/celllines/g (http://www.dsmz.de/human_and_animal_cell_lines/info.phpdsmz_nr=3&from=cell_line_index&select=H&term=&prese
HMEC	*human mammary epithelial cell*	human		epithelium	ECACC (http://www.hpacultures.org.uk/products/celllines/p
HT-29		human	colon epithelium	adenocarcinoma	ECACC (http://www.hpacultures.org.uk/products/celllines/g Cell Line Data Base (http://bioinformatics.istge.it/hypercldb/
Jurkat		human	T-Cell-Leukemia	white blood cells	ECACC (http://www.hpacultures.org.uk/products/celllines/g DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/dsmz_nr=282&from=cell_line_index&select=J&term=&pres
JY cells		human	lymphoblastoid	EBV immortalised B cell	
K562 cells		human	lymphoblastoid	CML blast crisis	ECACC (http://www.hpacultures.org.uk/products/celllines/g
Ku812		human	lymphoblastoid	erythroleukemia	ECACC (http://www.hpacultures.org.uk/products/celllines/g LGCstandards (http://www.lgcstandards-atcc.org/LGCAdva2099&Template=cellBiology)
KCL22		human	lymphoblastoid	CML	
KG1		human	lymphoblastoid	AML	
KYO1	Kyoto 1	human	lymphoblastoid	CML	DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/
	Lymph node Cancer of		prostatic		ECACC (http://www.hpacultures.org.uk/products/celllines/g

LNCap	the Prostate	human	adenocarcinoma	epithelial	(http://www.atcc.org/common/catalog/numSearch/numResul
Ma-Mel 1, 2, 3....48		human		a range of melanoma cell lines	
MC-38		mouse		adenocarcinoma	
MCF-10A	*Michigan Cancer Foundation*	human	mammary gland	epithelium	ATCC (http://www.lgcpromochem-atcc.com/common/catalo
MDA-231		human	breast	cancer	ECACC (http://www.hpacultures.org.uk/products/celllines/g
MDA-468		human	breast	cancer	ECACC (http://www.hpacultures.org.uk/products/celllines/g
MDA-MB-435		human	breast	melanoma or carcinoma (disputed)	Cambridge Pathology (http://www.path.cam.ac.uk/~pawefisl (http://www.hpacultures.org.uk/products/celllines/generalcel
MDCK II	*Madin Darby canine kidney*	dog	kidney	epithelium	ECACC (http://www.hpacultures.org.uk/products/celllines/g atcc.com/common/catalog/numSearch/numResults.cfm?atcc
MDCK II	*Madin Darby canine kidney*	dog	kidney	epithelium	[2] (http://www.hpacultures.org.uk/products/celllines/genera atcc.com/common/catalog/numSearch/numResults.cfm?atcc
MOR/0.2R		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
MONO-MAC 6		human	WBC	myeloid metaplasic AML	Cell Line Data Base (http://bioinformatics.istge.it/hypercldb/
MTD-1A		mouse		epithelium	
MyEnd	*myocardial endothelial*	mouse		endothelium	
NCI-H69/CPR		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
NCI-H69/LX10		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
NCI-H69/LX20		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
NCI-H69/LX4		Human	Lung		ECACC (http://www.hpacultures.org.uk/products/celllines/g
NIH-3T3	*NIH, 3-day transfer, inoculum 3 x 10^5 cells*	mouse	embryo	fibroblast	ECACC (http://www.hpacultures.org.uk/products/celllines/g atcc.com/common/catalog/numSearch/numResults.cfm?atcc
NALM-1			peripheral blood	blast-crisis CML	Cancer Genetics and Cytogenetics (http://www.sciencedirect C&_user=2471587&_rdoc=1&_fmt=&_orig=search&_sort=
NW-145				melanoma	ESTDAB (http://www.ebi.ac.uk/cgi-bin/ipd/estdab/print_cel
OPCN / OPCT cell lines	Onyvax[3] (http://www.onyvax.com/) Prostate Cancer....			Range of prostate tumour lines	Asterand (http://solutions.asterand.com/Human-Prostate-Cel
Peer		human	T cell leukemia		DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/ dsmz_nr=006&from=cell_line_index&select=search_for_ter
PNT-1A / PNT 2				Prostate tumour lines	ECACC (http://www.hpacultures.org.uk/products/celllines/g
RenCa	Renal Carcinoma	mouse		renal carcinoma	
RMA/RMAS		mouse		T cell tumour	
Saos-2 cells		human		Osteosarcoma	ECACC (http://www.hpacultures.org.uk/products/celllines/g
Sf-9	*Spodoptera frugiperda*	insect - *Spodoptera frugiperda* (moth)	Ovary		DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/
SkBr3		human		breast carcinoma	
T2		human		T cell leukemia/B cell line hybridoma	DSMZ (http://www.dsmz.de/human_and_animal_cell_lines/
T84		human	colorectal Carcinoma / lungmetastasis	epithelium	ECACC (http://www.hpacultures.org.uk/products/celllines/g atcc.com/common/catalog/numSearch/numResults.cfm?atcc
THP1 cell line		human	monocyte	AML	ECACC (http://www.hpacultures.org.uk/products/celllines/g
U373		human	glioblastoma-astrocytoma	epithelium	
U87		human	glioblastoma-astrocytoma	epithelial-like	Abcam (http://www.abcam.com/index.html?datasheet=1490

U937		human	leukaemic monocytic lymphoma		ECACC (http://www.hpacultures.org.uk/products/celllines/g
VCaP	Vertebra Prostate Cancer	human	metastatic prostate cancer	epithelial	ECACC (http://www.hpacultures.org.uk/products/celllines/g (http://www.atcc.org/ATCCAdvancedCatalogSearch/Produc
Vero cells	'Vera Reno' ('green kidney') / 'Vero' ('truth')	African Green Monkey	kidney epithelium		ECACC (http://www.hpacultures.org.uk/products/celllines/g
WM39		human	skin	primary melanoma	
WT-49		human	lymphoblastoid		
X63		mouse	melanoma		
YAC-1		mouse	lymphoma		Cell Line Data Base (http://bioinformatics.istge.it/hypercldb/ refId=86022801&collection=ecacc_gc)
YAR		human	B-cell	EBV transofrmed	[4] (http://www.hpacultures.org.uk/products/celllines/hlatyp (http://www.sciencedirect.com/science?_ob=ArticleURL&_t 2&_user=2471587&_rdoc=1&_fmt=&_orig=search&_sort=

Note: this list is a sample of available cell lines, and is not comprehensive

See also

- Biological immortality
- Cell culture assays
- List of contaminated cell lines
- Organ culture
- Plant tissue culture
- Tissue culture
- Electric cell-substrate impedance sensing

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14. ^ cold as vector news (http://wired.com/news/wireservice/0,70102-0.html?tw=wn_index_7)
15. ^ cold as vector research abstract (http://jvi.asm.org/cgi/content/abstract/80/4/1959)
16. ^ adjuvant (http://www3.niaid.nih.gov/news/newsreleases/2004/h9n2.htm)

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External links

- Health Protection Agency Culture Collections - including ECACC (European Collection of Cell Cultures) (http://www.hpacultures.org.uk/)
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- atcc (http://www.atcc.org/)
- Coriell Cell Repositories (http://ccr.coriell.org/)
- The National Centre for Cell Science (http://www.nccs.res.in/) (NCCS), Pune, India; national repository for cell lines/hybridomas etc.
- Neural Stem Cell Culture: Neurosphere generation, microscopical analysis and cryopreservation (a protocol) (http://www.natureprotocols.com/2006/08/25/neural_stem_cell_culture_neuro.php)
- Rat Chromaffin cells primary cultures: Standardization and quality assessment for single-cell assays (a protocol) (http://www.natureprotocols.com/2006/09/29/rat_chromaffin_cells_primary_c.php)
- Table of common cell lines from Alberts 4th ed. (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.table.1515)
- Cancer Cells in Culture (http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/CancerCellsInCulture.html)
- Hypertext version of the Cell Line Data Base (http://bioinformatics.istge.it/hypercldb/)